Form PTO-1390 (REV 10-2000) U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
0609.4730000	
U.S. APPLICATION NO (IF KNOWN, SEE 37 C.F.R. § 1.5)	
N/A	09/869565

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US98/27862	31 DECEMBER 1998	NONE

TITLE OF INVENTION
PTH RECEPTOR AND SCREENING ASSAY UTILIZING THE SAME

APPLICANT(S) FOR DO/EO/US
GARDELLA *et al.*

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 372(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: Authorization To Treat A Reply As Incorporating An Extension Of Time Under 37 C.F.R. § 1.136(a)(3) (*in duplicate*);
Application Data Sheet; and
Two (2) return postcards.

U.S. APPLICATION NO (if known, see 37 C.F.R. 1.50)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
N/A 09/869565	PCT/US98/27862	0609.4730000

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**	$ 690.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... **$ 100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 690.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	Number Filed		Number Extra	Rate		
Total Claims	19	- 20 =	0	X **$18.00**	$ 0.00	
Independent Claims	3	- 3 =	0	X **$80.00**	$ 0.00	
Multiple dependent claim(s) (if applicable)				+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =					$	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.					$	
SUBTOTAL =					$ 820.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +					$	
TOTAL NATIONAL FEE =					$ 820.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +					$	
TOTAL FEES ENCLOSED =					$ 820.00	
					Amount to be refunded:	$
					charged:	$

a. ☒ A check in the amount of $820.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0036. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit Under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

1100 New York Avenue, NW, Suite 600

Washington, D.C. 20005-3934

SIGNATURE

Steven R. Ludwig

NAME

36,203

REGISTRATION NUMBER

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*LIMITED TO MATTERS AND PROCEEDINGS BEFORE FEDERAL COURTS & AGENCIES
**REGISTERED PATENT AGENT
***SENIOR COUNSEL

October 17, 2001

WRITER'S DIRECT NUMBER:
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Box Missing Parts

Commissioner for Patents
Washington, D.C. 20231

Re: U.S. Utility Patent Application
Appl. No. 09/869,565; Int'l Filing Date: December 31, 1998
For: **PTH Receptor and Screening Assay Utilizing the Same**
Inventors: Gardella *et al.*
Our Ref: 0609.4730000/SRL/M-G

Sir:

In reply to the Notification of Missing Requirements Under 35 U.S.C. 371 in the United States Designated/Elected Office (DO/EO/US) dated **August 17, 2001**, Applicants submit the following documents for appropriate action by the U.S. Patent and Trademark Office:

1. Copy of the Notification of Missing Requirements Under 35 U.S.C. 371 in the United States Designated/Elected Office (DO/EO/US);

2. Copy of the Notification to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures;

3. Original Declaration, executed by the inventors;

4. Preliminary Amendment and Submission of Sequence Listing;

5. Paper and computer readable copy of Sequence Listing; and

6. One (1) return postcard.

It is respectfully requested that the attached postcard be stamped with the date of filing of these documents, and that it be returned to our courier.

In accordance with 37 C.F.R. § 1.821(f), the paper copy and the computer readable copy of the Sequence Listing submitted herewith are the same. In accordance with 37 C.F.R. § 1.821(g), the submission of this sequence listing includes no new matter.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036. If extensions of time under 37 C.F.R. § 1.136 other than those otherwise provided for herewith are required to prevent abandonment of the present patent application, then such extensions of time are hereby petitioned, and any fees therefor are hereby authorized to be charged to our Deposit Account No. 19-0036. A duplicate copy of this letter is enclosed.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Steven R. Ludwig
Attorney for Applicants
Registration No. 36,203

Enclosures

P:\USERS\MGRANOVS\0609\4730000\473 REPLY.NFMP TRANS WPD

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Gardella *et al.*	Art Unit: *To be assigned*
Appl. No. 09/869,565	Examiner: *To be assigned*
Int'l Filing Date: December 31, 1998	Atty. Docket: 0609.4730000/SRL/M-G
For: **PTH Receptor and Screening Assay Utilizing the Same**	

Preliminary Amendment and Submission of Sequence Listing

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Notification of Missing Requirements, dated August 17, 2001, in the above identified matter, and in advance of prosecution, please amend the application as follows:

In the Specification:

Please insert the sequence listing at the end of the application.

Remarks

No new matter has been added. The specification has been amended to direct the entry of this sequence listing after the claims of the above identified application and to provide the SEQ ID NO's next to the specific sequence.

In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application are the same.

Summary

It is respectfully believed that this application is now in condition for examination. Early notice to this effect is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Steven R. Ludwig
Attorney for Applicants
Registration No. 36,203

Date: 10/17/01

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Suite 600
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SEQUENCE LISTING

<110> Gardella, Thomas J.
Kronenberg, Henry M.
Potts Jr., John T.

<120> PTH Receptor and Screening Assay Utilizing the Same

<130> 0609.4730000

<140> 09/869,565
<141> 2001-06-29

<150> PCT/US98/27862
<151> 1998-12-31

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  1               5                  10                  15

cca gtg ctc agc tcc gca tat gcg ctg gag gta ttt gac cgc cta ggc   96
Pro Val Leu Ser Ser Ala Tyr Ala Leu Glu Val Phe Asp Arg Leu Gly
             20                  25                  30

atg atc tac acc gtg gga tac tcc atg tct ctc gcc tcc ctc acg gtg   144
Met Ile Tyr Thr Val Gly Tyr Ser Met Ser Leu Ala Ser Leu Thr Val
         35                  40                  45

gct gtg ctc atc ctg gcc tat ttt agg cgg ctg cac tgc acg cgc aac   192
Ala Val Leu Ile Leu Ala Tyr Phe Arg Arg Leu His Cys Thr Arg Asn
     50                  55                  60

tac atc cac atg cac atg ttc ctg tcg ttt atg ctg cgc gcc gcg agc   240
Tyr Ile His Met His Met Phe Leu Ser Phe Met Leu Arg Ala Ala Ser
 65                  70                  75                  80
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atc ttc gtg aag gac gct gtg ctc tac tct ggc ttc acg ctg gat gag   288
Ile Phe Val Lys Asp Ala Val Leu Tyr Ser Gly Phe Thr Leu Asp Glu
                85                  90                  95

gcc gag cgc ctc aca gag gaa gag ttg cac atc atc gcg cag gtg cca   336
Ala Glu Arg Leu Thr Glu Glu Glu Leu His Ile Ile Ala Gln Val Pro
            100                 105                 110

cct ccg ccg gcc gct gcc gcc gta ggc tac gct ggc tgc cgc gtg gcg   384
Pro Pro Pro Ala Ala Ala Ala Val Gly Tyr Ala Gly Cys Arg Val Ala
        115                 120                 125

gtg acc ttc ttc ctc tac ttc ctg gct acc aac tac tac tgg atc ctg   432
Val Thr Phe Phe Leu Tyr Phe Leu Ala Thr Asn Tyr Tyr Trp Ile Leu
    130                 135                 140

gtg gag ggg ctg tac ttg cac agc ctc atc ttc atg gcc ttt ttc tca   480
Val Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Phe Ser
145                 150                 155                 160

gag aag aag tac ctg tgg ggc ttc acc atc ttt ggc tgg ggt cta ccg   528
Glu Lys Lys Tyr Leu Trp Gly Phe Thr Ile Phe Gly Trp Gly Leu Pro
                165                 170                 175

gct gtc ttc gtg gct gtg tgg gtc ggt gtc aga gca acc ttg gcc aac   576
Ala Val Phe Val Ala Val Trp Val Gly Val Arg Ala Thr Leu Ala Asn
            180                 185                 190

act ggg tgc tgg gat ctg agc tcc ggg cac aag aag tgg atc atc cag   624
Thr Gly Cys Trp Asp Leu Ser Ser Gly His Lys Lys Trp Ile Ile Gln
        195                 200                 205

gtg ccc atc ctg gca tct gtt gtg ctc aac ttc atc ctt ttt atc aac   672
Val Pro Ile Leu Ala Ser Val Val Leu Asn Phe Ile Leu Phe Ile Asn
    210                 215                 220

atc atc cgg gtg ctt gcc act aag ctt cgg gag acc aat gcg ggc cgg   720
Ile Ile Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg
225                 230                 235                 240

tgt gac acc agg cag cag tac cgg aag ctg ctc agg tcc acg ttg gtg   768
Cys Asp Thr Arg Gln Gln Tyr Arg Lys Leu Leu Arg Ser Thr Leu Val
                245                 250                 255

ctc gtg ccg ctc ttt ggt gtg cac tac acc gtc ttc atg gcc ttg ccg   816
Leu Val Pro Leu Phe Gly Val His Tyr Thr Val Phe Met Ala Leu Pro
            260                 265                 270

tac acc gag gtc tca ggg aca ttg tgg cag atc cag atg cat tat gag   864
Tyr Thr Glu Val Ser Gly Thr Leu Trp Gln Ile Gln Met His Tyr Glu
        275                 280                 285
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atg ctc ttc aac tcc ttc cag gga ttt ttt gtt gcc atc ata tac tgt   912
Met Leu Phe Asn Ser Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys
    290                 295                 300

ttc tgc aat ggt gag gtg cag gca gag att agg aag tca tgg agc cgc   960
Phe Cys Asn Gly Glu Val Gln Ala Glu Ile Arg Lys Ser Trp Ser Arg
305                 310                 315                 320

tgg aca ctg gcg ttg gac ttc aag cgc aaa gca cga agt ggg agt agc   1008
Trp Thr Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Ser
                325                 330                 335

agc tac agc tat ggc cca atg gtg tct cac acg agt gtg acc aat gtg   1056
Ser Tyr Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val
            340                 345                 350

ggc ccc cgt gca gga ctc agc ctc ccc ctc agc ccc cgc ctg cct cct   1104
Gly Pro Arg Ala Gly Leu Ser Leu Pro Leu Ser Pro Arg Leu Pro Pro
        355                 360                 365

gcc act acc aat ggc cac tcc cag ctg cct ggc cat gcc aag cca ggg   1152
Ala Thr Thr Asn Gly His Ser Gln Leu Pro Gly His Ala Lys Pro Gly
    370                 375                 380

gct cca gcc act gag act gaa acc cta cca gtc act atg gcg gtt ccc   1200
Ala Pro Ala Thr Glu Thr Glu Thr Leu Pro Val Thr Met Ala Val Pro
385                 390                 395                 400

aag gac gat gga ttc ctt aac ggc tcc tgc tca ggc ctg gat gag gag   1248
Lys Asp Asp Gly Phe Leu Asn Gly Ser Cys Ser Gly Leu Asp Glu Glu
                405                 410                 415

gcc tcc ggg tct gcg cgg ccg cct cca ttg ttg cag gaa gga tgg gaa   1296
Ala Ser Gly Ser Ala Arg Pro Pro Pro Leu Leu Gln Glu Gly Trp Glu
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Thr Val Met
        435
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            20                  25                  30

Met Ile Tyr Thr Val Gly Tyr Ser Met Ser Leu Ala Ser Leu Thr Val
        35                  40                  45

Ala Val Leu Ile Leu Ala Tyr Phe Arg Arg Leu His Cys Thr Arg Asn
    50                  55                  60

Tyr Ile His Met His Met Phe Leu Ser Phe Met Leu Arg Ala Ala Ser
65                  70                  75                  80

Ile Phe Val Lys Asp Ala Val Leu Tyr Ser Gly Phe Thr Leu Asp Glu
                85                  90                  95

Ala Glu Arg Leu Thr Glu Glu Glu Leu His Ile Ile Ala Gln Val Pro
            100                 105                 110

Pro Pro Pro Ala Ala Ala Ala Val Gly Tyr Ala Gly Cys Arg Val Ala
        115                 120                 125

Val Thr Phe Phe Leu Tyr Phe Leu Ala Thr Asn Tyr Tyr Trp Ile Leu
    130                 135                 140

Val Glu Gly Leu Tyr Leu His Ser Leu Ile Phe Met Ala Phe Phe Ser
145                 150                 155                 160

Glu Lys Lys Tyr Leu Trp Gly Phe Thr Ile Phe Gly Trp Gly Leu Pro
                165                 170                 175

Ala Val Phe Val Ala Val Trp Val Gly Val Arg Ala Thr Leu Ala Asn
            180                 185                 190

Thr Gly Cys Trp Asp Leu Ser Ser Gly His Lys Lys Trp Ile Ile Gln
        195                 200                 205

Val Pro Ile Leu Ala Ser Val Val Leu Asn Phe Ile Leu Phe Ile Asn
    210                 215                 220

Ile Ile Arg Val Leu Ala Thr Lys Leu Arg Glu Thr Asn Ala Gly Arg
225                 230                 235                 240

Cys Asp Thr Arg Gln Gln Tyr Arg Lys Leu Leu Arg Ser Thr Leu Val
                245                 250                 255

Leu Val Pro Leu Phe Gly Val His Tyr Thr Val Phe Met Ala Leu Pro
            260                 265                 270

Tyr Thr Glu Val Ser Gly Thr Leu Trp Gln Ile Gln Met His Tyr Glu
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        275                 280                 285

Met Leu Phe Asn Ser Phe Gln Gly Phe Phe Val Ala Ile Ile Tyr Cys
    290                 295                 300

Phe Cys Asn Gly Glu Val Gln Ala Glu Ile Arg Lys Ser Trp Ser Arg
305                 310                 315                 320

Trp Thr Leu Ala Leu Asp Phe Lys Arg Lys Ala Arg Ser Gly Ser Ser
                325                 330                 335

Ser Tyr Ser Tyr Gly Pro Met Val Ser His Thr Ser Val Thr Asn Val
            340                 345                 350

Gly Pro Arg Ala Gly Leu Ser Leu Pro Leu Ser Pro Arg Leu Pro Pro
        355                 360                 365

Ala Thr Thr Asn Gly His Ser Gln Leu Pro Gly His Ala Lys Pro Gly
    370                 375                 380

Ala Pro Ala Thr Glu Thr Glu Thr Leu Pro Val Thr Met Ala Val Pro
385                 390                 395                 400

Lys Asp Asp Gly Phe Leu Asn Gly Ser Cys Ser Gly Leu Asp Glu Glu
                405                 410                 415

Ala Ser Gly Ser Ala Arg Pro Pro Pro Leu Leu Gln Glu Gly Trp Glu
            420                 425                 430

Thr Val Met
        435
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PTH Receptor and Screening Assay Utilizing the Same

Background of the Invention

Statement as to Rights to Inventions Made Under Federally-Sponsored Research and Development

Part of the work performed during development of this invention utilized U.S. Government funds. The U.S. Government has certain rights in this invention.

Field of the Invention

The present invention is related to the fields of molecular biology, developmental biology, physiology, neurobiology, endocrinology and medicine.

Related Art

PTH is the principal regulator of blood calcium levels and mediates this action through binding to PTH-1 receptors on bone and kidney cells (Kronenberg, H.M., *et al.*, in "Handbook of Experimental Pharmacology, Springer-Verlag," Heidelberg (1993)). This receptor also responds to PTH-related peptide, a factor which plays a role in embryonic bone development and is the causative agent of hypercalcemia of malignancy (Lanske, B., *et al., Science 273*:663-666 (1996)). PTH and PTHrP peptides have been shown to have potent anabolic effects on bone, and it is possible, therefore, that PTH-1 receptor agonists could ultimately be used to treat metabolic bone diseases, such as osteoporosis (Dempster, D.W., *et al., Endocr Rev. 14(6)*:690-709 (1994)).

In the fully bioactive PTH(1-34) peptide, the major determinants of receptor-binding affinity reside within amino acids 15 to 34 (Nussbaum, S.R., *et al., J. Biol. Chem. 255*:10183-10187 (1980); Gardella, T.J., *et al., Endocrinology*

132(5):2024-2030 (1993); Caulfield, M.P., *et al.*, *Endocrinology 127*:83-87 (1990); Abou-Samra, A. B., *et al.*, *Endocrinology 125*:2215-2217 (1989)), which are moderately conserved among PTHs and PTHrPs from various species (Suva, L.J., *et al.*, *Science 237(4817)*:893-896 (1987)). The determinants of receptor activation lie within the more stringently conserved amino-terminal residues, and deletion of these residues yields competitive PTH-1 receptor antagonists (Horiuchi, N., *et al.*, *Science 220*:1053-1055 (1983); Nutt, R.F., *et al.*, *Endocrinology 127*:491-493 (1990)). Amino-terminal PTH or PTHrP fragments shorter in length than PTH(1-27) have not previously been found to be biologically active (Rosenblatt, M., *Pathobilogy Annual*, Raven Press, New York, 11:53-84 (1981); Azarani, A., *et al.*, *J. Biol. Chem. 271(25)*:14931-14936 (1996); Tregear, G.W., *et al.*, *Endocrinology 93*:1349-1353 (1973)), yet the functional importance and evolutionary conservation of the amino-terminal residues predicts that they directly interact with the receptor.

The PTH-1 receptor couples strongly to the adenylyl cyclase/protein kinase A signaling pathway and, in some settings, to other pathways including those mediated by phospholipase C/protein kinase C and intracellular calcium (About-Samra, A. B., *et al.*, *Endocrinology 129*:2547-2554 (1991); Jüppner, H., *et al.*, *Science 254*:1024-1026 (1991); Guo, J. *et al.*, *Endocrinology 136*:3884-3891 (1995); Hruska, K.A., *et al.*, *J. Clin. Invest. 79*:230-239 (1987); Donahue, H.J., *et al.*, *J. Biol. Chem. 263*:13522-13527 (1988)). The PTH-1 receptor is a member of the family B subgroup of G protein-coupled receptors, which also includes the receptors for calcitonin and secretin (Kolakowski, L. F., "GCRDb: A G-Protein-Coupled Receptor Database," *Receptors and Channels 2*:1-7 (1994)). Mutagenesis and crosslinking studies have indicated that multiple domains of these receptors contribute to ligand interaction, including the large amino-terminal extracellular domain, the extracellular loops and the transmembrane helices (Jüppner, H., *et al.*, *Endocrinology 134*:879-884 (1994); Lee, C., *et al.*, *Mol. Endo. 9*:1269-1278 (1995); Turner, P., *et al.*, *J. Bone Min. Res. 12(1)*:Abstract 121 (1997); Dautzenberg, F., *et al.*, *Proc. Natl. Acad. Sci.*

95:4941-4946 (1998); Holtmann, M., *et al*., *J Biol. Chem 270*:14394-14398 (1995); DeAlmeida, V. and Mayo, K., *Mol. Endo. 12*:750-765 (1998); Stroop, S., *et al., Biochem. 34*:1050-1057 (1994); Zhou, A., *et al., Proc. Natl. Acad. Sci. USA 94*:3644-3649 (1997); Bisello, A., *et al., J. Biol. Chem. 273*:22498-22505 (1998)). Studies using PTH/calcitonin chimeric receptors and hybrid ligands have suggested a general topology of the interaction in which the amino-terminal extracellular domain of the receptor recognizes the carboxyl-terminal binding domain of the ligand, while the "core" region of the receptor containing the seven transmembrane helices and connecting loops recognizes the amino-terminal signaling portion of the ligand (Bergwitz, C., *et al., J. Biol. Chem. 271*:26469-26472 (1996)). Similar conclusions were derived from earlier receptor chimera studies (Jüppner, H., *et al., Endocrinology 134*:879-884 (1994); Stroop, S., *et al., Biochem. 34*:1050-1057 (1994); Gardella, T.J., *et al., Endocrinology 135*:1186-1194 (1994)) and from recent crosslinking studies with photoreactive PTH analogs (Bisello, A., *et al., J. Biol. Chem. 273*:22498-22505 (1998); Mannstadt, M., *et al., J. Biol. Chem. 273*:16890-16896 (1998)).

In the current study we investigate the signaling component of the interaction between PTH and the PTH-1 receptor using a domain-based approach. This approach employs short amino-terminal PTH fragment analogs and a PTH receptor mutant that lacks most of the amino-terminal extracellular domain. The results of cAMP-signaling assays performed with these smaller ligands and receptors demonstrate that the conserved amino-terminal (Kronenberg, H.M., *et al.*, in "Handbook of Experimental Pharmacology, Springer-Verlag," Heidelberg (1993); Lanske, B., *et al., Science 273*:663-666 (1996); Dempster, D.W., *et al., Endocr Rev. 14(6)*:690-709 (1994); Nussbaum, S.R., *et al., J. Biol. Chem. 255*:10183-10187 (1980); Gardella, T. J., *et al., Endocrinology 132(5)*:2024-2030 (1993); M. P. Caulfield *et al., Endocrinology 127*:83-87 (1990); A. B. Abou-Samra *et al., Endocrinology 125*:2215-2217 (1989); Suva, L.J., *et al., Science 237(4817)*:893-896 (1987); Horiuchi, N., *et al., Science 220*:1053-1055 (1983); Nutt, R. F., *et al., Endocrinology 127*:491-493 (1990); Rosenblatt, M.,

Pathobilogy Annual, Raven Press, New York, 11:53-84 (1981); Azarani, A., *et al.*, *J. Biol Chem. 271(25)*:14931-14936 (1996); Tregear, G.W., *et al.*, *Endocrinology 93*:1349-1353 (1973); About-Samra, A.B., *et al.*, *Endocrinology 129*:2547-2554 (1991)) segment of PTH functions as an autonomous signaling domain and that this domain interacts with the core region of the receptor.

Summary of the Invention

PTH is the principal regulator of blood calcium levels and mediates this action through binding to PTH-1 receptors on bone and kidney cells. PTH-1 receptor agonists may ultimately be used to treat metabolic bone diseases, such as osteoporosis. Thus there is a strong need in the art develop new and improved PTH and PTH receptor reagents for the treatment of human disease.

In a first embodiment, the invention provides a novel PTH-1 receptor polypeptide, rδNt, characterized by a deletion of the extracellular amino-terminal ligand binding domain. The invention also provides nucleic acid molecules encoding the rδNt receptor polypeptide.

In a second embodiment the rδNt receptor of the invention is useful for screening procedures designed to identify agonists and antagonists of PTH receptor function. The invention provides screening utilizing either cAMP accumulation or competitive binding for the evaluation of test compounds with cells expressing the rδNt receptor.

Brief Description of the Figures

Figure 1. Presentation of the nucleic acid sequence and the amino acid sequence of the mutant PTH1R receptor, rδNt.

Figure 2. cAMP-stimulating Activity of PTH Fragments in LLC-PK1 Cells. A) Rat PTH(1-34) analog or amino-terminal rPTH fragments were tested for cAMP-stimulating activity in an LLC-P1-derived cell line (HKRK-B7) stably

transfected with the human PTH-1 receptor. Cells were treated with peptide at the indicated doses for 60 min at 22°C. Intracellular cAMP was measured by RIA, as described in Experimental Procedures. Shown are combined data (mean ± s.e.m.) from 3 separate experiments, each performed in duplicate. B) HKRK-B7 cells, or untransfected LLC-PK1 were treated with rPTH(1-34) or rPTH(1-14), and intracellular cAMP was measured. Shown are data (mean ± s.e.m.) from a single representative experiment performed in duplicate.

Figure 3. Alanine-scan of PTH(1-14). HKRK-B7 cells were treated with 100 mM of one of 14 different rPTH(1-14) analogs, each having a different alanine-substitution at the indicated amino acid position. The resulting cAMP levels were determined as described in Experimental Procedures. Shown are the combined data (mean ±s.e.m.) from three separate experiments, each performed in duplicate. The mean (mean ±s.e.m.) basal cAMP levels observed in the three experiments was 2. 1 ±0.1 pmole/well, and the maximum response to rPTH(1-34) at 0.1 mM was 254± 16 pmole/well.

Figure 4. PTH Responses of Intact and Truncated PTH-1 Receptors in COS-7 Cells. Shown at the top are schematics of the intact (A) and truncated (B) rat PTH-1 receptors used for transient transfection of COS-7 cells, and subsequent cAMP response assays. The conserved extracellular cysteine residues are depicted as open circles and numbered according to sequence position, and the nine amino acids of the epitope tag (HA) in rWT-HA are shaded. The tics at residue 26 and 181 indicate the endpoints of the deletion in rδNt. Based on the predicted signal peptide cleavage site at Ala-22, residues 23-25 in rδNt are joined to residue 182. The cAMP responses of COS-7 cells expressing the intact receptor (C) and rδNt (D) to rPTH(1-34) (J) or rPTH(1-14) (C) are also shown. The graphs show combined data (mean ±s.e.m.) from five separate experiments, each performed in duplicate.

Figure 5. Alanine-scan of PTH(1-14) with Intact and Truncated PTH Receptors. COS-7 cells transiently transfected with rWT-HA (A) or rCE I -G (B) were treated with 100 mM of native rat PTH(1-14) or 100 mM of a rPTH(1-14)

analog containing a single alanine substitution for 1h at 21 °C, and the resulting intracellular cAMP levels were measured by RIA. The amino acid substitutions are indicated on the axis labels. Peptides were tested in duplicate, and a single experiment representative of three others is shown.

Figure 6. Specificity of the Truncated Ligand and PTH Receptor. COS-7 cells transiently transfected with either rWT-HA (A), rδNt (B), or the intact rat secretin receptor (C), were treated with the indicated peptides for 60 min at 22 °C, and the resulting intracellular cAMP levels were quantified by RIA. Concentration of peptides present during the incubations were: rPTH(1-34), 0.1 mM; rPTH(1-14) 100 mM; secretin(1-27), 1 mM and secretin(1-13), 100 mM. Shown are data (mean ± s.e.m.) from one experiment performed in duplicate, and this was repeated twice more with equivalent results.

Figure 7. Antagonist Properties of PTHrP(7-34) with PTH(1-14). COS-7 cells transfected with rWT-HA (A) or rδNt (B) were treated with the antagonist [Leu11,D-Trp12]hPTHrP(7-34)NH2 (or buffer alone), for 5 min at 22°C, followed by 10 ml of either rPTH(1-34) or rPTH(1-14) agonist peptide. Incubations were continued for 30 min at 21 °C and the resulting cAMP levels were measured by RIA, as described in Experimental Procedures. The final concentration of antagonist peptide present during the incubation was 10 mM. Shown are data from a single experiment performed in triplicate. A repeat of the same experiment yielded equivalent results.

Detailed Description of the Preferred Embodiments

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the rδNt receptor polypeptide, a novel, mutant PTH1R receptor polypeptide, having the amino acid sequence shown in Figure 1 (SEQ ID NO:2), which was determined by sequencing a cloned cDNA. The rδNt protein of the present invention shares sequence homology with previously identified non-mutant PTH1R and PTH2R sequences. The nucleotide sequence shown in Figure

1 (SEQ ID NO:1) was obtained by sequencing a cDNA clone (rδNt), which was deposited on ____ at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and given accession number ____.

1. The rδNt Receptor

a) Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined by manual sequencing, and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by manual sequencing are typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Using the information provided herein, such as the nucleotide sequence in Figures 1, a nucleic acid molecule of the present invention encoding a rδNt polypeptide, respectively, may be obtained using standard techniques. Cloning and screening procedures are known for the isolation of the wild-type PTH1R sequence, such as those for cloning cDNAs using mRNA as starting material. Subsequent to cloning the wild-type receptor, the appropriate deletion in the sequence may be made as described herein. Illustrative of the invention, the nucleic acid molecule described in Figure 1 (SEQ ID NO:1) was obtained by using standard restriction enzyme digestion and cloning techniques in the art. The determined nucleotide sequence of the rδNt cDNA of Figure 1 (SEQ ID NO:1)

contains an open reading frame encoding a protein of about 435 amino acid residues, with a predicted leader sequence of about 22 amino acid residues. The amino acid sequence of the predicted mature rδNt receptor is shown in Figure 1 from amino acid residue about 23 to residue about 435. The rδNt protein shown in Figure 1 (SEQ ID NO:2) is about 84% identical to the rat PTH1 receptor.

As indicated, the present invention also provides the mature form(s) of the rδNt receptor of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature rδNt polypeptides having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. ____ and as shown in Figure 1 (SEQ ID NO:2). By the mature rδNt protein having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit ____ is meant the mature form(s) of the rδNt receptor produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature rδNt receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____ may or may not differ from the predicted "mature" rδNt protein shown in Figure 1 (amino acids from about 23 to about 435) depending on the accuracy of the predicted cleavage.

Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the

methods of McGeoch (*Virus Res. 3*:271-286 (1985)) and von Heinje (*Nucleic Acids Res. 14*:4683-4690 (1986)) can be used. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. von Heinje, *supra*. However, the two methods do not always produce the same predicted cleavage point(s) for a given protein. A computational method may be found in the computer program "PSORT" (K. Nakai and M. Kanehisa, Genomics 14:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated.

In the present case, the predicted amino acid sequence of the complete rδNt polypeptide of the present invention was analyzed for structural properties by comparison to the rat rδNt sequence. This analysis provided predicted a cleavage site between amino acids 22 and 23 in Figure 1 (SEQ ID NO:2). Thus, the leader sequence for the rδNt receptor protein is predicted to consist of amino acid residues 1-22 in Figure 1 (amino acids 1 to 22 in SEQ ID NO:2) , while the predicted mature rδNt protein consists of residues 23-435 (amino acids 23 to 435 in SEQ ID NO:2).

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

As one of ordinary skill would appreciate, however, due to the possibilities of sequencing errors, the rδNt receptor polypeptide encoded by the deposited cDNA comprises about 435 amino acids, but may be anywhere in the range of 425-435 amino acids; and the leader sequence of this protein is about 22 amino acids, but may be anywhere in the range of about 10 to about 30 amino acids.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in Figure 1 (SEQ ID NO:1); DNA molecules comprising the coding sequence for the rδNt receptor shown in Figure 1 (SEQ ID NO:2); and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the rδNt receptor. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

In another aspect, the invention provides isolated nucleic acid molecules encoding the rδNt polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. ____ on ____. Preferably, the nucleic acid molecule will encode the mature polypeptide encoded by the above-described deposited cDNA clone. In a further embodiment, a nucleic acid molecule is provided encoding the rδNt polypeptide or the rδNt polypeptide lacking the N-terminal methionine. The invention also provides an

isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1 or the nucleotide sequence of the rδNt cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the rδNt gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNAs or the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments of about 50-1550 nt in length, and more preferably at fragments least about 600 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNAs or as shown in Figure 1 (SEQ ID NO:1). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNAs or the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1).

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising the rδNt receptor extracellular domain (predicted to constitute amino acid residues from about 23 to about 147 in Figure 1 (or amino acid residues from about 23 to about 147 in SEQ ID NO:2)); a polypeptide comprising the rδNt receptor transmembrane domain (predicted to constitute amino acid residues from about 148 to about 416 in Figure 1 (or amino acid residues from about 148 to about 416 in SEQ ID NO:2)); and a polypeptide comprising the rδNt receptor extracellular domain with all or part of the transmembrane domain deleted. As above with the leader sequence,

the amino acid residues constituting the rδNt receptor extracellular and transmembrane domains have been predicted. Thus, as one of ordinary skill would appreciate, the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding epitope-bearing portions of the rδNt receptor protein. As one skilled in the art would know, a nucleic acid sequence may be used to predict the polypeptide sequence encoded therein. Such information may then be used to predict antigenic determinants in the polypeptide that may be related to the corresponding polynucleotide regions encoding the antigenic determinants identified by the analysis. Methods for predicting the antigenic determinants of a polypeptide are well known in the art.

Methods for determining other such epitope-bearing portions of the rδNt protein are described in detail below.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clones contained in ATCC Deposit Nos._____ or _____. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNAs or the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1).

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) tract of the rδNt receptor cDNA shown in Figure 1 (SEQ ID NO:1), or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode a rδNt polypeptide may include, but are not limited to those encoding the amino acid sequence of the mature polypeptides, by themselves; the coding sequence for the mature polypeptides and additional sequences, such as those encoding the amino acid leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5′ and 3′ sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc Natl. Acad. Sci. USA 86*:821-824 (1989), for instance, hexa-histidine provides for

convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al.*, *Cell 37*: 767 (1984). As discussed below, other such fusion proteins include the rδNt receptor fused to Fc at the amino or C-terminus.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the rδNt receptor. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions, which may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the rδNt receptor or portions thereof. Also especially preferred in this regard are conservative substitutions.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95%, 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the full-length rδNt polypeptide having the complete amino acid sequence in SEQ ID NO:2, including the predicted leader sequence; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the mature rδNt receptor (full-length polypeptide with the leader removed) having the amino acid sequence at positions from about 23 to about 435 in SEQ ID NO:2; (d) a nucleotide sequence encoding the full-length rδNt polypeptide having the

complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No.____; (e) a nucleotide sequence encoding the mature rδNt receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97883; (f) a nucleotide sequence encoding the rδNt receptor extracellular domain; (g) a nucleotide sequence encoding the rδNt receptor transmembrane domain; (h) a nucleotide sequence encoding the rδNt receptor extracellular domain with all or part of the transmembrane domain deleted; and (i) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g) or (h).

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a rδNt polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the rδNt receptor. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5′ or 3′ terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figure 1 or to the nucleotides sequence of the deposited cDNA clones can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive,

Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNAs, irrespective of whether they encode a polypeptide having rδNt receptor activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having rδNt receptor activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having rδNt receptor activity include, *inter alia*, (1) isolating the rδNt receptor gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the rδNt receptor gene, as described in Verma *et al.*, *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting rδNt receptor mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNA which do, in fact, encode a polypeptide having rδNt receptor activity. By "a polypeptide having rδNt receptor activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the rδNt receptor of

the invention, as measured in a particular biological assay. For example, rδNt receptor activity can be measured using competition binding experiments of labeled PTH or PTHrP to cells expressing the candidate rδNt polypeptide as described herein.

Any cell line expressing the rδNt receptor, or variants thereof, may be used to assay ligand binding and second messenger activation as described in Examples 3 and 4. Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNAs or the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) will encode a polypeptide "having rδNt receptor activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having rδNt protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247*:1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

b) Vectors and Host Cells

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically

engineered with the recombinant vectors, and the production of rδNt polypeptides or fragments thereof by recombinant techniques.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac, trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli, Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1

and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al.*, *Basic Methods In Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such

as. hIL5-receptor has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See. D. Bennett *et al.*, *Journal of Molecular Recognition*, Vol. 8:52-58 (1995) and K. Johanson *et al.*, *The Journal of Biological Chemistry*, Vol. 270. No. 16:9459-9471 (1995).

The rδNt receptor can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

c) rδNt Polypeptides and Fragments

The invention further provides an isolated rδNt polypeptide having the amino acid sequence encoded by the deposited cDNAs, or the amino acid sequence in Figure 1 (SEQ ID NO:2) or a peptide or polypeptide comprising a portion of the above polypeptides.

It will be recognized in the art that some amino acid sequences of the rδNt receptor can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine

activity. Thus, the invention further includes variations of the rδNt receptor which show substantial rδNt receptor activity or which include regions of rδNt protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990).

Thus, the fragment, derivative or analog of the polypeptide of Figure 1 (SEQ ID NO:2) or that encoded by the deposited cDNAs, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the rδNt protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al.*, *Clin Exp. Immunol. 2*:331-340 (1967); Robbins *et al.*, *Diabetes*

36:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993)).

The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al.*, *Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-α to only one of the two known types of TNF receptors. Thus, the rδNt receptor of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in the rδNt protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations

at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al., J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the antimicrobial peptide polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988).

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the rδNt receptor can be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988).

The polypeptides of the present invention also include the polypeptide encoded by the deposited rδNt cDNA including the leader, the polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein), the polypeptide of Figure 1 (SEQ ID NO:2) including the leader, the polypeptide of Figure 1 (SEQ ID NO:2) minus the leader, the extracellular domain, the transmembrane domain, a polypeptide comprising amino acids about 1 to about 435 in SEQ ID NO:2, and a polypeptide comprising amino acids about 2 to about 435 in SEQ ID NO:2, as well as polypeptides which are at least 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a rδNt polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the rδNt receptor. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figure 1 (SEQ ID NO:2) to the amino acid sequence encoded by deposited cDNA clones can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

2. *Agonists and Antagonists of the rδNt Receptor Activity*

Functional characterization of the biological properties of the rδNt receptor of the invention and derivatives thereof may be performed by bioassays that measure ligand-stimulated cAMP accumulation.

A. Assay for the Detection of Cyclic AMP Accumulation in Cells Expressing rδNt Receptor After Exposure to Test Compounds

Intracellular cAMP accumulation is measured as described previously (Abou-Samra *et al., J. Biol. Chem. 262*:1129, 1986). Cells expressing the rδNt receptor grown in 24-well plates are rinsed with culture medium containing 0.1% BSA and 2 mM IBMX. The cells are then incubated with a test compound for 60 min. at 21 °C. The supernatant is removed and the cells immediately frozen by placing the whole plate in dry ice powder. Intracellular cAMP is extracted by thawing the cells in 1 ml of 50 mM HCl and analyzed by a specific radioimmunoassay using an anti-cAMP antibody (e.g., Sigma, St. Louis, Mo). A cAMP analog (2'-O-monosuccinyl-adenosine 3':5'-cyclic monophosphate tyrosyl methyl ester, obtained from Sigma) which is used a tracer for cAMP is iodinated by the chloramine T method. Free iodine is removed by adsorbing the iodinated cAMP analog onto a C18 Sep-pak cartridge (Waters, Milford, Mass.). After washing with dH_20, the iodinated cAMP analog is eluted from the Sep-pak Cartridge with 40% acetonitrille (ACN) and 0.1% trifluoroacetic acid (TFA). The iodinated cAMP analog is lyophilized, reconstituted in 1 ml 0.1% TFA, and injected into a C18 reverse phase HPLC column (Waters). The column is equilibrated with 10% ACN in 0.1% TFA, and eluted with gradient of 10-30% ACN in 0.1% TFA. This allows separation of the mono-iodinated cAMP analog from the non-iodinated cAMP analog. The tracer is stable for up to 4 months when stored at - 20 °C. The standard used for the assay, adenosine 3':5'-cyclic monophosphate, may be purchased from Sigma. Samples (1-10 82 l of HCl extracts) or standards (0.04-100 fmol/tube) are diluted in 50 mM Na-acetate (pH 5.5), and acetylated with 10 µl of mixture of triethylamine and acetic anhydride (2:1 vol:vol). After acetylation, cAMP antiserum (100 µl) is added from a stock

solution (1:4000) made in PBS (pH 7.4), 5 mM EDTA and 1% normal rabbit serum. The tracer is diluted in PBS (pH 7.4) with 0.1% BSA, and added (20,000 cpm/tube). The assay is incubated at 4°C. overnight. The bound tracer is precipitated by adding 100 µl of goat anti-rabbit antiserum (1:20 in PBS) and 1 ml of 7% polyethyleneglycol (MW 5000-6000), centrifuging at 2000 rpm for 30 min. at 4°C. The supernatant is removed and the bound radioactivity is counted in a gamma-counter (Micromedic). To compute the cAMP data, logit calculations are performed in Excel spreadsheets. Typically, the assay sensitivity is 0.1 fmol/tube, and the standard concentration that displaces 50% of tracer is 5 fmol/tube.

B. Screening Compounds Utilizing an rδNt Receptor Binding Assay

In addition to the cAMP accumulation assay described below, it is possible that compounds may be iodinated and used in a radioreceptor-based assay in rδNt transiently transfected COS cells. COS-7 cells are grown in 15 cm plates in DMEM, 10% heat-inactivated FBS, 10 mg/L gentamycin until 80-90% confluent. Twenty-four hours after transfection by the DEAE/Dextran method (Sambrook *et al., supra*), with 1-2µg of plasmid DNA, the cells are trypsinized and replated in multiwell plastic dishes (16 or 35 mm diameter, Costar, Cambridge, Mass.) at a cell concentration of 5×10^4 cells/cm^2. Cell number increased only slightly after transfection. After continuing culture for another 48 h, radioreceptor assays are performed. The culture medium is replaced with buffer containing 50 mM Tris-HCL (pH 7.7), 100 mM NaCl, 2 mM $CaCl_2$, 5 mM KCL, 0.5% heat-inactivated fetal bovine serum (GIBCO), and 5% heat-inactivated horse serum (KC Biological Inc., Lenexa, Kans.) immediately before studies are initiated. Unless otherwise indicated, studies are conducted with cells incubated in this buffer at 15°C. for 4 h with 4×10^5 cpm/ml (9.6×10^{-11} M) of ^{125}I-labeled [Ala^1]PTH(1-14) amide or ^{125}I-labeled [Nle^8]PTH(1-14).

C. *Screening for PTH-1 Receptor Antagonists and Agonists*

The rδNt receptor of the invention may be utilized in to screen for compounds that are agonistic or antagonistic to the PTH response using the cAMP accumulation assay. Cells expressing PTH-1 receptor on the cell surface are incubated with native PTH(1-84) for 5-60 minutes at 37°C., in the presence of 2 mM IBMX (3-isobutyl-1-methyl-xanthine, Sigma, St. Louis, MO). Cyclic AMP accumulation is measured by specific radio-immunoassay, as described above. A test compound that competes with native PTH(1-84) for binding to the rδNt receptor, and that inhibits the effect of native PTH(1-84) on cAMP accumulation, is considered a competitive antagonist. Such a compound would be useful for treating hypercalcemia.

Conversely, a test compound that does not compete with native PTH(1-84) for binding to the rδNt receptor, but which still prevents native PTH(1-84) activation of cAMP accumulation (presumably by blocking the receptor activation site) is considered a non-competitive antagonist. Such a compound would be useful for treating hypercalcemia.

A candidate compound that competes with native PTH(1-84) for binding to the rδNt receptor, and which stimulates cAMP accumulation in the presence or absence of native PTH(1-84) is a competitive agonist. A candidate compound that does not compete with native PTH(1-84) for binding to the rδNt receptor but which is still capable of stimulating cAMP accumulation in the presence or absence of native PTH(1-84), or which stimulates a higher cAMP accumulation than that observed with a PTH compound would be considered a non-competitive agonist.

Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to PTH or PTHrP. The method involves contacting cells which express the rδNt polypeptide with a candidate compound and the PTH or PTHrP ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with

the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or PTH or PTHrP (e.g., cyclic AMP accumulation). By the invention, a cell expressing the rδNt polypeptide can be contacted with either an endogenous or exogenously administered PTH or PTHrP.

Examples

General Methods

Peptides: Peptides were prepared by the Biopolymer Synthesis Facility at Massachusetts General Hospital (Boston, MA) using solid-phase chemistry with Fmoc (N-(9-fluorenyl)methoxycarbonyl) protecting groups, and TFA-mediated cleavage and deprotection. All peptides were C-terminally amidated. The PTH(1-14) analogs were synthesized on a multiple peptide synthesizer (Advanced Chemtech Model 396 MBS) at 0.025 mM scale. The completed peptides were desalted by adsorption on a C18 cartridge (Sep-Pak) and then analyzed by reversed-phase C18-based HPLC, MALDI-mass spectromety and amino acid analysis. The PTH(1-34) control peptide, [$Nle^{8,21}$,Tyr^{34}]rPTH-(1-34)NH_2, and the PTHrP(7-34) antagonist peptide, [Leu^{11},D-Trp^{12}]hPTHrP(7-34)NH_2, were prepared on an Applied Biosystems Synthesizer (Model 431A) 0.1 mM scale, purified by reversed-phase C18-based HPLC and characterized as described above. Concentrated stock solutions of peptides, 10 mM for PTH(1-14) analogs and 0.3 mM for PTHrP(7-34) and PTH(1-34), were prepared in 10 mM acetic acid, quantified by acid hydrolysis and amino acid analysis and stored at -80°C.

Cell Culture and DNA Transfection: COS-7 and HKRK-B7 cells were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10%); penicillin G (20 units/ml), streptomycin sulfate (20

μg/ml) and amphotericin B (0.05 μg/ml) in a humidified atmosphere containing 5% CO_2. Twenty-four to 16 hours prior to assay, cells in 24-well plates were shifted to a humidified incubator containing 5% CO_2 that was set at 33°C. Stock solutions of EGTA/trypsin and antibiotics were from GIBCO; fetal bovine serum was from Hyclone Laboratories (Logan, UT). Derivation and characterization of the HKRK-B7 cell line by stable transfection of LLC-PK_1 cells with a pCDNA-1-based plasmid (In Vitrogen, San Diego, CA) encoding the hPTH-1 receptor was described previously (Takasu, H. and Bringhurst, F., *Endocrinology*, in press (1998)). These cells express PTH-1 receptors at a surface density of about $1x10^6$ PTH-binding sites per cell. The HKRK-B7 cells were used for functional assays 24 to 72 hours after the cell monolayer reached confluency.

For studies with the intact and truncated rat PTH-1 receptors, transient transfections of COS-7 cells were performed using DEAE-dextran as described previously (Bergwitz, C., *et al.*, *J. Biol. Chem. 272*:28861-28868)). The construction and initial characterization of the pCDNA-1 based plasmids encodin either the intact or truncated rat PTH-1 receptor has been described previously (Lee, C., *et al.*, *Endocrinology 135(4)*:1488-1495)). The intact receptor (rWT-HA) contains a nine amino acid HA epitope tag in place of residues 93-101 of the extracellular domain; this epitope tag does not affect receptor function (Lee, C., *et al.*, *Endocrinology 135(4)*:1488-1495)). The truncated rat PTH-1 receptor (rδNt) is deleted for exons E1 through exon G (residues 26 to 181). Assuming that signal peptidase cleavage occurs between Ala-22 and Tyr-23 (Nielsen, H., *et al.*, *Protein Engineering 10*:1-6 (1997)), rδNt is predicted to have for its N-terminus residues Dautzenberg, F., *et al.*, *Proc. Natl. Acad. Sci. 95*:4941-4946 (1998); Holtmann, M., *et al.*, *J. Biol. Chem. 270*:14394-14398 (1995); DeAlmeida, V. and Mayo, K., *Mol. Endo. 12*:750-765 (1998)) joined to Glu-182 (Figure 3B). COS-7 cells were transfected in 24-well plates when the cells were 85 to 95% of confluency, using 200 ng of plasmid DNA that was purified by cesium chloride/ethidium bromide gradient centrifugation for each well. Assays were conducted 72 to 96 hours after transfection. Under these conditions about

~20 % of the COS-7 cells become transfected and express about $5x10^6$ surface PTH receptors per cell (Bergwitz, C., *et al.*, *J. Biol. Chem.* 272:28861-28868)).

Intracellular cyclic AMP: Transfected COS-7 or HKRK-B7 cells were rinsed with 500 ml of binding buffer, (50 mM Tris-HCl, pH 7.7, 100 mM NaCl, 5 mM KCl, 2 mM CaC12, 5% heat-inactivated horse serum, 0.5% heat-inactivated fetal bovine serum) and 200 ml of IBMX buffer (DMEM containing 2 mM IBMX, 1 mg/ml bovine serum albumin, 35 mM Hepes-NaOH, pH 7.4) and 100 ml of binding buffer or binding buffer containing various amounts of peptide were added. The plates were incubated for 60 minutes at room temperature. The buffer was then withdrawn and the cells were frozen on dry ice, treated with 0.5 ml of 50 mM HCl, and refrozen. After the thawing, the lysate was diluted 30-fold in dH20 and an aliquot was analyzed for cAMP content by determined radioimmunoassay using unlabeled cAMP as a standard.

For cAMP inhibition assays, transfected COS-7 cells were rinsed once with 500 ml of binding buffer, and 200 ml of IBMX buffer and 100 ml of binding buffer or binding buffer containing the antagonist [Leu11,D-Trp12]hPTHrP(7-34) NH2 (10 mM) were added. After a 5 minute incubation at room temperature, 10 ml of binding buffer containing PTH(1-14) or PTH(1-34) (agonist peptide) was added, and the incubation was continued for an additional 30 minutes. The cells were then lysed and intracellular cAMP levels were measured as described above.

Example 1

PTH(1-14) action in stable cells.

Amino-terminal peptide fragments based on the rat PTH sequence and ranging in length from PTH(1-9) to PTH(1-15) were synthesized and tested for activity in an LLC-PK1-derived cell line called HKRK-B7 which stably expresses high levels ($1x10^6$ receptors/cell) of the cloned human PTH-1 receptor (Takasu, H. and Bringhurst, F., *Endocrinology*, in press (1998)). As shown in Figure 1A, the intact control peptide PTH(1-34) mediated a 50-fold increase in intracellular cAMP levels relative to the basal cAMP level, and the estimated EC50 for this

response was ~2nM with PH(1-13)and shorter fragments little or no increase in cAMP accumulation was observed (Figure 1A). However, two of the amino-terminal fragments, PTH(1-14) and PTH(1-15), stimulated cAMP formation to about 20-fold over the basal level, although the doses required for this activation were five to six orders of magnitude higher than the dose required for PTH(1-34). The response to these active peptides was dependent on the transfected PTH receptor, as parental LLC-PK1 cells, which do not express PTH receptors, but do express the related calcitonin receptor, were unresponsive to PTH(1-34) or PTH(1-14) (Figure 2B).

With the intact receptor, the potency of PTH(1-14) was about five orders of magnitude weaker than that of PTH(1-34). This reduced potency is not surprising, given that the PTH(1-14) peptide lacks important receptor-binding residues located in the PTH(15-34) region in the PTH(15-34) domain (Nussbaum, S.R., *et al.*, *J. Biol. Chem. 255*:10183-10187 (1980); Gardella, T.J., *et al.*, *Endrocrinology 132(5)*:2024-2030 (1993); Caulfield, M.P., *et al.*, *Endocrinology 127*:83-87 (1990); and Abou-Samra, A.-B., *et al.*, *Endocrinology 125*:2215-2217 (1989)). Consistent with this, unlabeled PTH(1-14) bound too weakly to permit detection in our standard competition binding assays which used radioiodinated rPTH(1-34) as a tracer ligand, nor could we detect direct binding of radiolabled PTH(1-14) analog to the intact or truncated receptors used in this study (data not shown).

Example 2

Alanine Scanning of PTH(1-14)

To identify residues in the PTH(1-14) fragment that play a role in activating the adenylyl cyclase-signaling pathway, an alanine-scanning approach was employed. Thirteen different alanine-substituted rat PTH(1-14) analogs were synthesized and tested for the ability to stimulate cAMP formation in HKRK-B7 cells (Figure 2). The activity profile obtained with the monosubstituted analogs revealed that residues in the 1-9 region formed a relatively intolerant segment of

the peptide, whereas residues in the 10-14 region formed a comparatively tolerant segment. Thus, with the exception of Ser-3 and Ala-1 (which is the native amino-terminal residue of rat PTH) most alanine substitutions in the 1-9 region yielded peptides that were barely active or inactive. In contrast, each alanine substitution in the 10-14 region yielded peptides with activities comparable with that of native rat PTH(1-14). The activity of the alanine-3 substituted peptide correlates well with previous studies on PTH(1-34) analogs which showed that amino acids with small side chains are tolerated at this site (Cohen, F.E., *et al., J. Biol. Chem. 266*:1997-2004 (1991)).

Example 3

Potency of the rδNt Receptor with Small Ligands

Experiments utilized COS-7 cells transfected with either the intact rat PTH-1 receptor (rWT-HA, Fig. 4A) or a truncated rat PTH-1 receptor with most of the amino-terminal extracellular domain deleted (rδNt, Fig. 4B). In COS-7 cells expressing rWT-HA, PTH(1-34) and PTH(1-14) mediated cAMP responses that were similar to the responses seen in HKRK-B7 cells: PTH(1-14) stimulated a 15-fold in cAMP formation, but with a potency that was four to five orders of magnitude weaker than that of PTH(1-34) (Figure 3C). Both peptides also stimulated cAMP formation in cells transfected with rδNt, but the potency of PTH(1-14) was only two orders of magnitude weaker than that of PTH(1-34) with this truncated receptor (Figure 3 D). This change in the relative potency of the two ligands could be accounted for by a 100-fold decrease in the potency which PTH(1-34) exhibited with rδNt, as compared to its potency with rWT-HA, rather than a shift in the potency of PTH(1-14), which was equipotent with the two receptors (compare panels C and D of Figure 4).

Although there was no direct measurement of receptor expression in this study, the 100-fold reduction in potency that PTH(1-34) exhibited with rδNt, as compared to rWT-HA, is not likely to be due to a reduction in surface expression of the truncated receptor, since PTH(1-14) exhibited equivalent activity with rδNt

and rWT-HA (Figure 4, C and D). This suggests that the two receptors are expressed at approximately equal levels. The reduced activity of PTH(1-34) with rδNt therefore most likely reflects a loss of important binding interactions that normally occur between the (Jüppner, H., *et al., Science 254*:1024-1026 (1991), Guo, J., *et al., Endocrinology 136*:3884-3891 (1995); Hruska, K.A., *J. Clin. Invest. 79*:230-239 (1987); Donahue, H.J., *et al., J. Biol. Chem. 263*:13522-13527 (1988); Kolakowski, L.F., GCRDb: *A G-protein–coupled receptor Database Receptors and Channels 2*:1-7 (1994); Jüppner, H., *et al., Endocrinology 134*:879-884 (1994); Lee, C., *et al., Mol. Endo. 9*:1269-1278 (1995); Turner, P., *et al.*, Single Mutations Allow the PTH-2 Receptor to Respond to PTHrP J. Bone Min. Res. 12, Supplement 1, Abstract #121 (1997); Dautzenberg, F., *et al., Proc. Natl. Acad. Sci. 95*:4941-4946 (1998), Holtmann, M., *et al., J. Biol. Chem. 270*:14394-14398 (1995); DeAlmeida, V., *et al., Mol. Endo. 12*:750-765 (1998); Stroop, S., *et al., Biochem. 34*:1050-1057 (1994); Zhou, A., *et al., Proc. Natl. Acad. Sci. USA 94*:3644-3649 (1997); Bisello, A., *et al., J. Biol Chem. 273*:22498-22505 (1998); Bergwitz, C., *et al., J. Biol. Chem. 271*:26469-26472 (1996); Gardella, T.J., *et al., Endocrinology 135*:1186-1194 (1994); Mannstadt, M., *et al., J. Biol. Chem. 273*:16890-16896 (1998); Takasu, H. and Bringhurst, F., *Endocrinology*, in press (1998); Bergwitz, C., *et al., J. Biol. Chem. 272*:28861-28868 (1997); Lee, C., *et al., Endocrinology 135(4)*:1488-1495 (1994)) domain of the ligand and the amino-terminal domain of the receptor (Jüppner, H., *et al., Endocrinology 134*:879-884 (1994); Bergwitz, C., *et al., J. Biol. Chem. 271*:16469-26472 (1996); Mannstadt, M., *et al., J. Biol. Chem. 273*:16890-16896 (1998)). The lack of these same binding interactions could also explain the inability of PTHrP(7-34) to function as an antagonist with rδNt (see Figure 7B).

Example 4:

The rδNt Receptor Interacts with the same PTH(1-14) Functional Residues as Intact PTH-1 receptor

Experiments were designed to test whether the PTH(1-14) residues that are required for function with the truncated receptor differ from those required for function with the intact receptor. Using the alanine-scanning set of PTH(1-14) analogs, experiments tested cAMP-stimulating activity for the two rat PTH-1 receptors in COS-7 cells. As shown in Figures 5A and 5B, the activity profiles obtained with rδNt mirrored that obtained with rWT-HA, since Ser-3 and the 10-14 region of the peptide were tolerant of mutation, whereas residues 2 and 4-9 were intolerant (Figures 4A and B). Therefore, the same set of functional residues in PTH(1-14) that are required for interaction with the intact PTH-1 receptor are also required for interaction with the core domain of the receptor.

Example 5

Specificity of Truncated Ligands and PTH-receptors

In order to test whether PTH(1-14) and rδNt retained the appropriate recognition specificity for the corresponding parent ligand, cross-reactivity experiments were done using secretin ligands and the cloned rat secretin receptor. COS-7 cells transfected with the secretin receptor exhibited a 50-fold increase in cAMP levels in response to secretin(1-27) (1 mM), but did not respond to either PTH(1-34) (1 mM) or PTH(1-14) (100 mM) (Figure 5C). Cells expressing rδNt responded to PTH(1-34) and PTH(1-14) but not to secretin(1-27) (1 mM) or secretin(1-13) (1 mM) (Figure 5B). Thus, the recognition specificity of PTH(1-14) and rδNt appear to replicate that of the intact parent molecules. No evidence for relaxed specificity was detected in these studies (Figure 6). It is also worth noting that PTH(1-14) did not activate the endogenous calcitonin receptors expressed in LLC-PK1 cells (Figure 2B).

Example 6

rδNt Stimulation is Not Affected by the Inhibitor [Leu11,D-Trp12]hPTHrP(7-34)NH2

In order to determine whether [Leu11,D-Trp12]hPTHrP(7-34)NH2, a potent competitive antagonist of PTH(1-34) action (Nutt, R.F., *et al.*, *Endocrinology 127*:491-493 (1990)), could block the ability of PTH(1-14) to stimulate cAMP formation in COS-7 cells expressing either rWT-HA or rδNt (Figure 6). With rWT-HA the inhibitor peptide reduced the efficacy of both PTH(1-14) and PTH(1-34) by as much as 70 % as compared to the responses elicited by these agonists in the absence of inhibitor (Figure 6A). In contrast, PTHrP(7-34) had little or no effect on the ability of PTH(1-34) or PTH(1-14) to stimulate cAMP production in cells expressing rδNt.

The ability of PTHrP(7-34) to antagonize PTH(1-14) action on the intact receptor (Figure 6A) suggests that the receptor sites occupied by these two ligands overlap. This overlap could involve ligand residues 7-14 and some portion in the core region of the receptor. Any binding interactions that may occur between figure residues (7-34) and the core region of the receptor are, however, too weak to enable effective antagonism in the absence of the amino-terminal extracellular receptor domain.

These findings establish that a much smaller region of PTH(1-34) than heretofore appreciated can stimulate receptor activation, and that the amino-terminal portion of PTH the hormone interacts with the core region of the receptor containing the seven transmembrane helices and connecting loops, as previously hypothesized for intact PTH ligands and receptors (Lee, C., *et al.*, *Mol. Endo. 9*:1269-1278 (1995); Bisello, A., *et al.*, *J. Biol. Chem. 273*:22498-22505 (1998); Bergwitz, C., *et al.*, *J. Biol. Chem. 271*:26469-26472 (1996); Gardella, T.J., *et al.*, *Endocrinology 135*:1186-1194 (1994); Bergwitz, C., *et al.*, *J. Biol. Chem. 272*:28861-28868; Gardella, T., *et al.*, *J. Biol. Chem. 271*:12820-12825 (1996)). Furthermore, this component of the interaction is sufficient for receptor signaling. The hypothesis that the 15-34 region of PTH binds to the amino-terminal extracellular domain of the receptor does not exclude the possibility that this domain, which by itself does not stimulate cAMP formation (data not shown), also provides some binding energy by interacting with the core region of the

receptor. In fact, the ~100-fold greater potency that PTH(1-34) exhibits with rδNt, in comparison to the potency of PTH(1-14) with this receptor (Figure 4D), might well be due to such interactions. However, we can not exclude the alternative possibility that the 15-34 domain enhances the intrinsic signaling activity of the (1-14) segment, for example, by stabilizing a favorable secondary structure in the amino-terminal portion of the ligand. More specific information on the receptor recognition sites utilized by PTH and the structure of the receptor-bound ligand are required to distinguish between such possibilities.

Some recognition determinants have been identified in the amino-terminal extracellular domain, the extracellular loops and the transmembrane helices of the B family of receptors (Turner, P., *et al.*, Single Mutations Allow the PTH-2 Receptor to Respond to PTHrP J. Bone Min. Res. 12, Supplement 1, Abstract #121 (1997), Dautzenberg, F., *et al.*, *Proc. Natl. Acad. Sci. 95*:4941-4946 (1998), Holtmann, M., *et al.*, *J. Biol. Chem. 270*:14394-14398 (1995), Gardella, T.J., *et al.*, *Endrocrinology 135*:1186-1194 (1994); Bergwitz, C., *et al.*, *J. Biol. Chem. 272*:28861-28868; Turner, P.R., *et al.*, *J. Biol. Chem. 271(16)*:9205-9208 (1996)).

One distinguishing feature of the family B receptors is the amino-terminal extracellular domain, which is relatively large and contains a number of conserved residues, including six cysteines. It is thus intriguing that this domain of the PTH-1 receptor is not essential for ligand-dependent signal transduction, as evidenced by the results with the rδNt receptor.

Several other reports on other family B receptors provide additional evidence to suggest that the amino-terminal extracellular domains of these receptors may not be essential for functional expression. Large amino-terminal deletions in the calcitonin receptor (Unson, C., *et al.*, *J. Biol. Chem. 270*:27720-27727 (1995)) and in the growth hormone-releasing factor receptor (DeAlmedia, V. and Mayo, K., *Mol. Endo. 12*:750-765 (1998)) were compatible with expression, as assessed by immunologic methods, and a glucagon receptor lacking the amino-terminal domain and containing an activating mutation in helix 2 (HR-

178) exhibited constitutive cAMP-signaling activity (Hjorth, S., *et al., Mol. Endo. 12*:78-86 (1998)). In these studies, however, evidence that the truncated receptor could interact with ligand, as we have found for the PTH-1 receptor, was not reported. In a separate study on the lutropin receptor, a group A receptor that binds the large glycohormone human choriogonadotropin, it was observed that a deletion of the large amino-terminal extracellular domain yielded a receptor that could mediate a cAMP response to high doses of hCG (Ji, I.H. and Ji, T.H., *J. Biol. Chem. 266(20)*:13076-13079 (1991)).

That the activity of PTH(1-14) was not affected by the deletion of the amino-terminal receptor domain suggests that the peptide interacts predominantly with the core region of the receptor. This conclusion is supported by the alanine-scanning experiments performed on PTH(1-14), in which the profile of tolerant and intolerant residues observed with rδNt was nearly the same as that obtained with the intact receptor (Figure 4). With each receptor, residues in the 10-14 region of the ligand formed a tolerant segment, while residues in the 1-9 region, excluding 1 and 3, formed an intolerant segment. This pattern of critical and non-critical residues observed in the context of the PTH(1-14) fragment closely matches the patterns found previously in studies on longer-length PTH analogs (Cohen, F.E., *et al., J. Biol. Chem. 266*:1997-2004 (1991); Gombert, F., *et al.*, in "Peptides: Chemistry, Structure and Biology Proceedings of the 14th American Peptide Symposium June 18-23, Kaumaya, P. and Hodges, R., eds., pp. 661-662, *Mayflower Scientific Limited*, Kingswinford, UK (1996); Gardella, T.J., *et al., J. Biol. Chem. 266*:13141-13146 (1991)).

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the rδNt receptor having the complete amino acid sequence at positions from about 1 to about 435 in SEQ ID NO:2;

(b) a nucleotide sequence encoding the rδNt receptor having the amino acid sequence at positions from about 2 to about 435 in SEQ ID NO:2;

(c) a nucleotide sequence encoding the mature rδNt receptor having the amino acid sequence at positions from about 23 to about 435 in SEQ ID NO:2;

(d) a nucleotide sequence encoding the rδNt receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____;

(e) a nucleotide sequence encoding the mature rδNt receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____;

(f) a nucleotide sequence encoding the rδNt extracellular domain;

(g) a nucleotide sequence encoding the rδNt transmembrane domain;

(h) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g).

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence in Figure 1 (SEQ ID NO:1).

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the rδNt receptor having the complete amino acid sequence in Figure 1 (SEQ ID NO:2).

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the mature rδNt receptor having the amino acid sequence in Figure 1 (SEQ ID NO:2).

5. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. ____.

6. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the rδNt receptor having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____.

7. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the mature rδNt receptor having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____.

8. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f) or (g) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

9. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a rδNt receptor having an amino acid sequence in (a), (b), (c), (d), (e), (f) or (g) of claim 1.

10. The isolated nucleic acid molecule of claim 1, which encodes the rδNt receptor extracellular domain.

11. The isolated nucleic acid molecule of claim 1, which encodes the rδNt receptor transmembrane domain.

12. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

13. A recombinant vector produced by the method of claim 12.

14. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 13 into a host cell.

15. A recombinant host cell produced by the method of claim 14.

16. A recombinant method for producing a rδNt polypeptide, comprising culturing the recombinant host cell of claim 15 under conditions such that said polypeptide is expressed and recovering said polypeptide.

17. An isolated rδNt polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the rδNt polypeptide having the complete amino acid sequence at positions from about 1 to about 435 in SEQ ID NO:2;

(b) the amino acid sequence of the rδNt polypeptide having the amino acid sequence at positions from about 2 to about 435 in SEQ ID NO:2;

(c) the amino acid sequence of the mature rδNt polypeptide having the amino acid sequence at positions from about 23 to about 435 in SEQ ID NO:2;

(d) the amino acid sequence of the rδNt polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____; and

(e) the amino acid sequence of the mature rδNt polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. ____.

18. An isolated antibody that binds specifically to a rδNt receptor polypeptide of claim 17.

19. A method of screening for agonists or antagonists of PTH receptor activity comprising:

(a) contacting a test compound to cells expressing a PTH receptor; and

(b) measuring the biological response of said cells.

```
       ATGGGGGCCGCCCGGATCGCACCCAGCCTGGCGCTCCTACTCTGCTGCCCAGTGCTCAGC
a      M  G  A  A  R  I  A  P  S  L  A  L  L  L  C  C  P  V  L  S  -

       TCCGCATATGCGCTGGAGGTATTTGACCGCCTAGGCATGATCTACACCGTGGGATACTCC
a      S  A  Y  A  L  E  V  F  D  R  L  G  M  I  Y  T  V  G  Y  S  -

       ATGTCTCTCGCCTCCCTCACGGTGGCTGTGCTCATCCTGGCCTATTTTAGGCGGCTGCAC
a      M  S  L  A  S  L  T  V  A  V  L  I  L  A  Y  F  R  R  L  H  -

       TGCACGCGCAACTACATCCACATGCACATGTTCCTGTCGTTTATGCTGCGCGCCGCGAGC
a      C  T  R  N  Y  I  H  M  H  M  F  L  S  F  M  L  R  A  A  S  -

       ATCTTCGTGAAGGACGCTGTGCTCTACTCTGGCTTCACGCTGGATGAGGCCGAGCGCCTC
a      I  F  V  K  D  A  V  L  Y  S  G  F  T  L  D  E  A  E  R  L  -

       ACAGAGGAAGAGTTGCACATCATCGCGCAGGTGCCACCTCCGCCGGCCGCTGCCGCCGTA
a      T  E  E  E  L  H  I  I  A  Q  V  P  P  P  P  A  A  A  A  V  -

       GGCTACGCTGGCTGCCGCGTGGCGGTGACCTTCTTCCTCTACTTCCTGGCTACCAACTAC
a      G  Y  A  G  C  R  V  A  V  T  F  F  L  Y  F  L  A  T  N  Y  -

       TACTGGATCCTGGTGGAGGGGCTGTACTTGCACAGCCTCATCTTCATGGCCTTTTTCTCA
a      Y  W  I  L  V  E  G  L  Y  L  H  S  L  I  F  M  A  F  F  S  -

       GAGAAGAAGTACCTGTGGGGCTTCACCATCTTTGGCTGGGGTCTACCGGCTGTCTTCGTG
a      E  K  K  Y  L  W  G  F  T  I  F  G  W  G  L  P  A  V  F  V  -

       GCTGTGTGGGTCGGTGTCAGAGCAACCTTGGCCAACACTGGGTGCTGGGATCTGAGCTCC
a      A  V  W  V  G  V  R  A  T  L  A  N  T  G  C  W  D  L  S  S  -

       GGGCACAAGAAGTGGATCATCCAGGTGCCCATCCTGGCATCTGTTGTGCTCAACTTCATC
a      G  H  K  K  W  I  I  Q  V  P  I  L  A  S  V  V  L  N  F  I  -

       CTTTTTATCAACATCATCCGGGTGCTTGCCACTAAGCTTCGGGAGACCAATGCGGGCCGG
a      L  F  I  N  I  I  R  V  L  A  T  K  L  R  E  T  N  A  G  R  -
```

FIG. 1

FIG. 1- CONTINUED

```
      TGTGACACCAGGCAGCAGTACCGGAAGCTGCTCAGGTCCACGTTGGTGCTCGTGCCGCTC
a     C  D  T  R  Q  Q  Y  R  K  L  L  R  S  T  L  V  L  V  P  L  -

      TTTGGTGTGCACTACACCGTCTTCATGGCCTTGCCGTACACCGAGGTCTCAGGGACATTG
a     F  G  V  H  Y  T  V  F  M  A  L  P  Y  T  E  V  S  G  T  L  -

      TGGCAGATCCAGATGCATTATGAGATGCTCTTCAACTCCTTCCAGGGATTTTTTGTTGCC
a     W  Q  I  Q  M  H  Y  E  M  L  F  N  S  F  Q  G  F  F  V  A  -

      ATCATATACTGTTTCTGCAATGGTGAGGTGCAGGCAGAGATTAGGAAGTCATGGAGCCGC
a     I  I  Y  C  F  C  N  G  E  V  Q  A  E  I  R  K  S  W  S  R  -

      TGGACACTGGCGTTGGACTTCAAGCGCAAAGCACGAAGTGGGAGTAGCAGCTACAGCTAT
a     W  T  L  A  L  D  F  K  R  K  A  R  S  G  S  S  S  Y  S  Y  -

      GGCCCAATGGTGTCTCACACGAGTGTGACCAATGTGGGCCCCCGTGCAGGACTCAGCCTC
a     G  P  M  V  S  H  T  S  V  T  N  V  G  P  R  A  G  L  S  L  -

      CCCCTCAGCCCCCGCCTGCCTCCTGCCACTACCAATGGCCACTCCCAGCTGCCTGGCCAT
a     P  L  S  P  R  L  P  P  A  T  T  N  G  H  S  Q  L  P  G  H  -

      GCCAAGCCAGGGGCTCCAGCCACTGAGACTGAAACCCTACCAGTCACTATGGCGGTTCCC
a     A  K  P  G  A  P  A  T  E  T  E  T  L  P  V  T  M  A  V  P  -

      AAGGACGATGGATTCCTTAACGGCTCCTGCTCAGGCCTGGATGAGGAGGCCTCCGGGTCT
a     K  D  D  G  F  L  N  G  S  C  S  G  L  D  E  E  A  S  G  S  -

      GCGCGGCCGCCTCCATTGTTGCAGGAAGGATGGGAAACAGTCATGTGACTGGGCACTAGG
3579  -+---------+---------+---------+---------+---------+--------  3638
a     A  R  P  P  P  L  L  Q  E  G  W  E  T  V  M  *              -
```

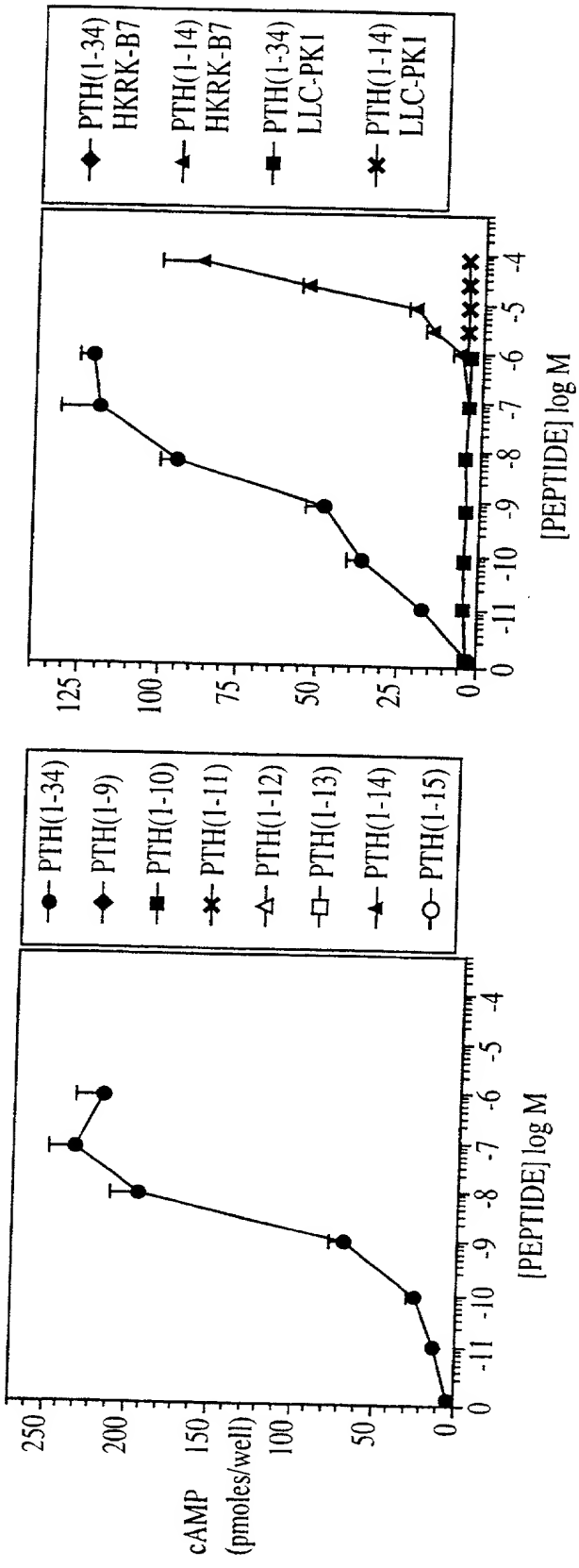
cAMP (pmoles/well)
250
200
150
100
50
0
-11 -10 -9 -8 -7 -6 -5 -4
[PEPTIDE] log M
PTH(1-34)
PTH(1-9)
PTH(1-10)
PTH(1-11)
PTH(1-12)
PTH(1-13)
PTH(1-14)
PTH(1-15)
125
100
75
50
25
0
-11 -10 -9 -8 -7 -6 -5 -4
[PEPTIDE] log M
PTH(1-34) HKRK-B7
PTH(1-14) HKRK-B7
PTH(1-34) LLC-PK1
PTH(1-14) LLC-PK1

FIG. 2A

FIG. 2B

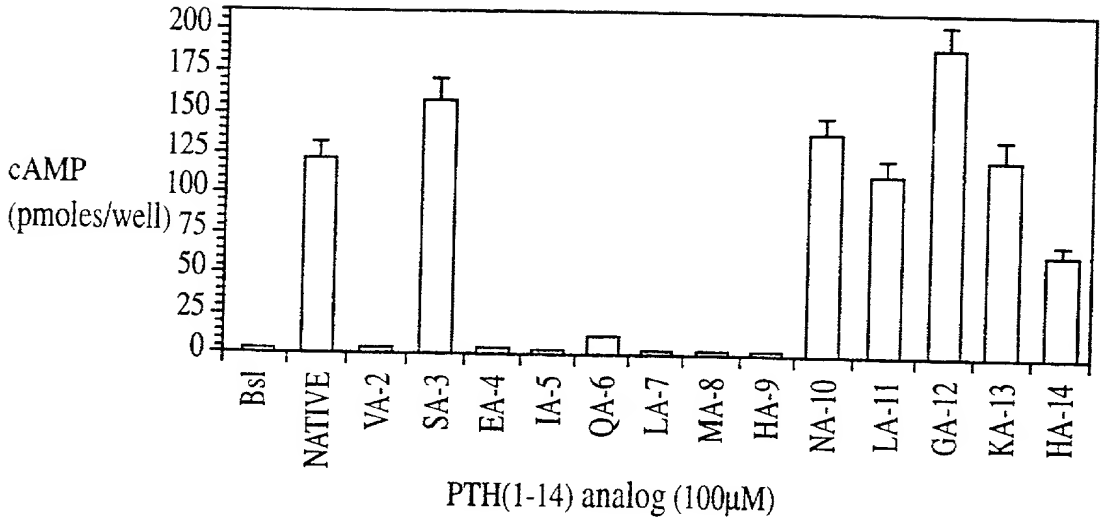
cAMP
(pmoles/well)
200
175
150
125
100
75
50
25
0
Bsl
NATIVE
VA-2
SA-3
EA-4
IA-5
QA-6
LA-7
MA-8
HA-9
NA-10
LA-11
GA-12
KA-13
HA-14
PTH(1-14) analog (100μM)

FIG. 3

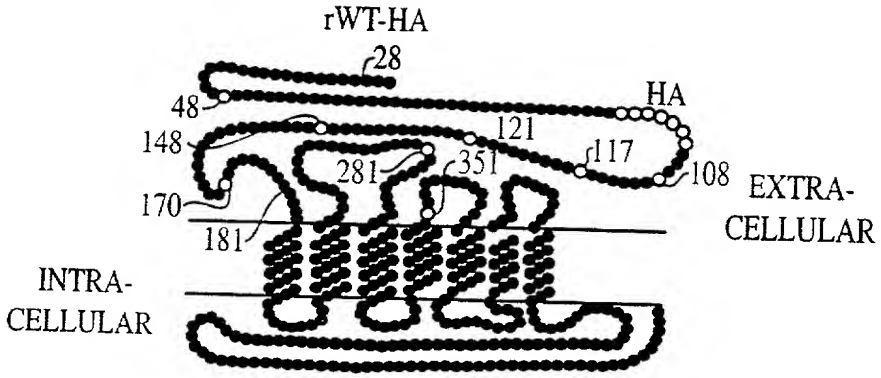

FIG. 4A

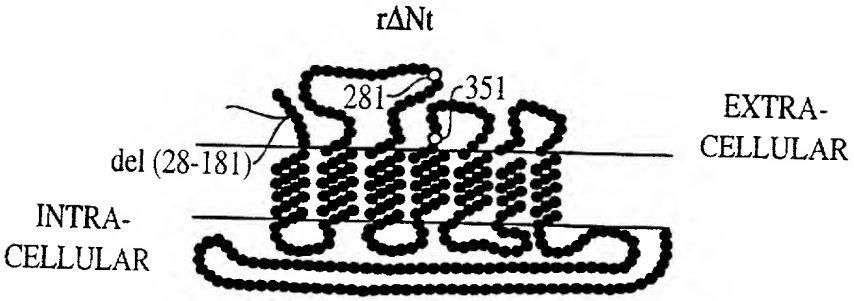

FIG. 4B

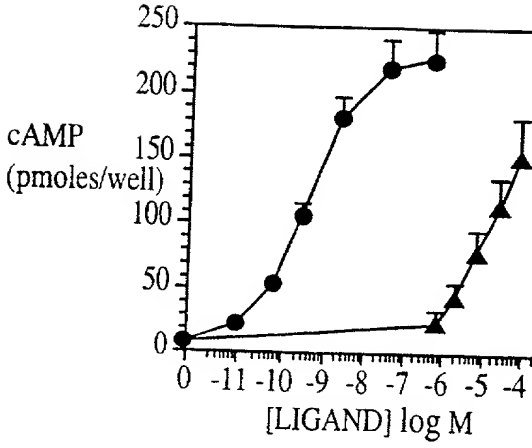

FIG. 4C

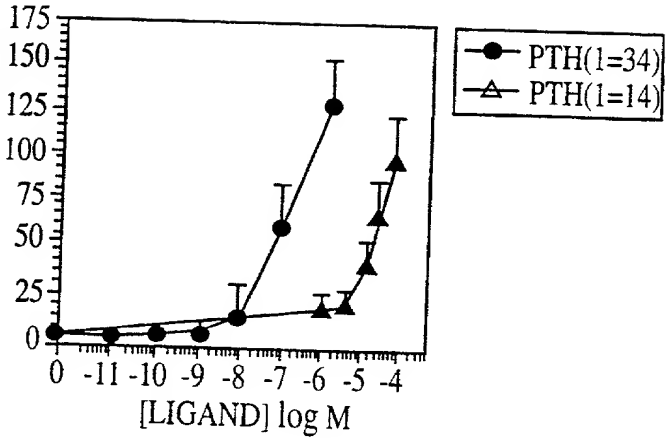

FIG. 4D

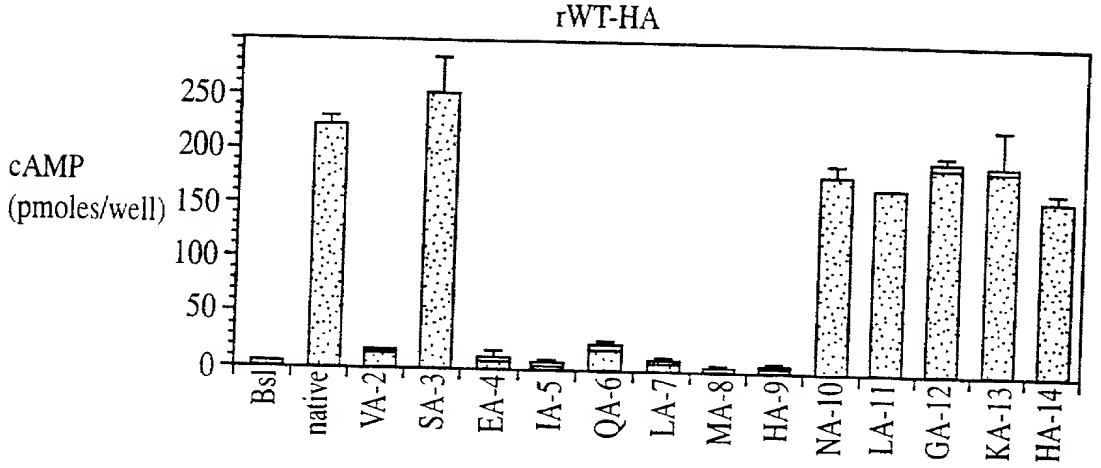
rWT-HA
cAMP
(pmoles/well)
250
200
150
100
50
0
Bsl
native
VA-2
SA-3
EA-4
IA-5
QA-6
LA-7
MA-8
HA-9
NA-10
LA-11
GA-12
KA-13
HA-14

FIG. 5A

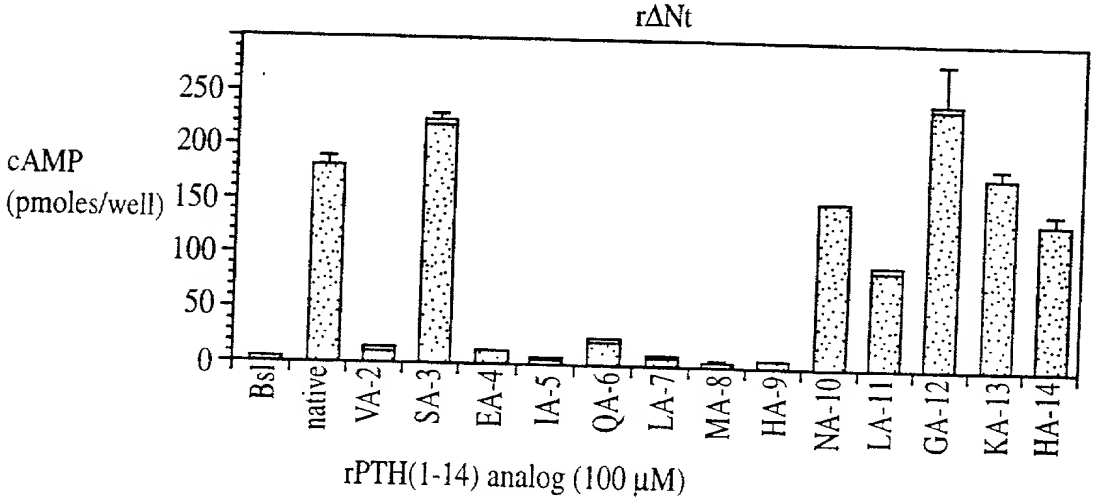
rΔNt
cAMP
(pmoles/well)
250
200
150
100
50
0
Bsl
native
VA-2
SA-3
EA-4
IA-5
QA-6
LA-7
MA-8
HA-9
NA-10
LA-11
GA-12
KA-13
HA-14
rPTH(1-14) analog (100 μM)

FIG. 5B

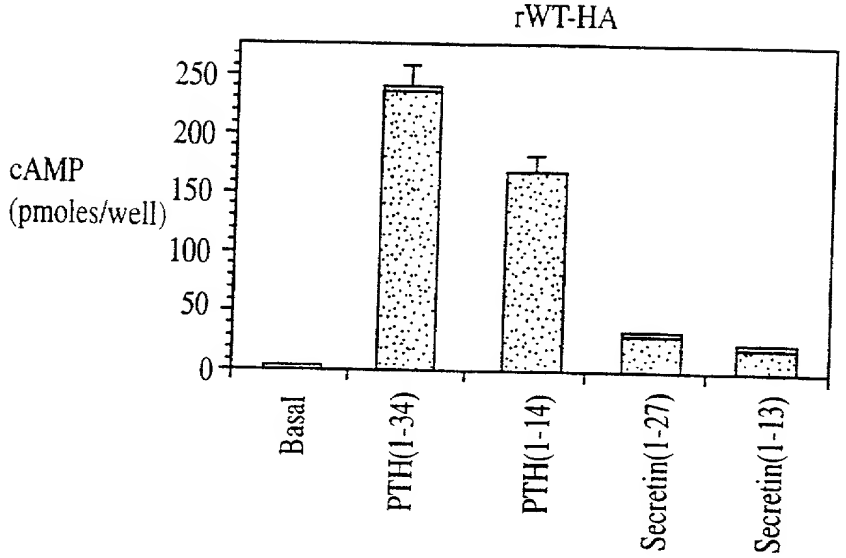

FIG. 6A

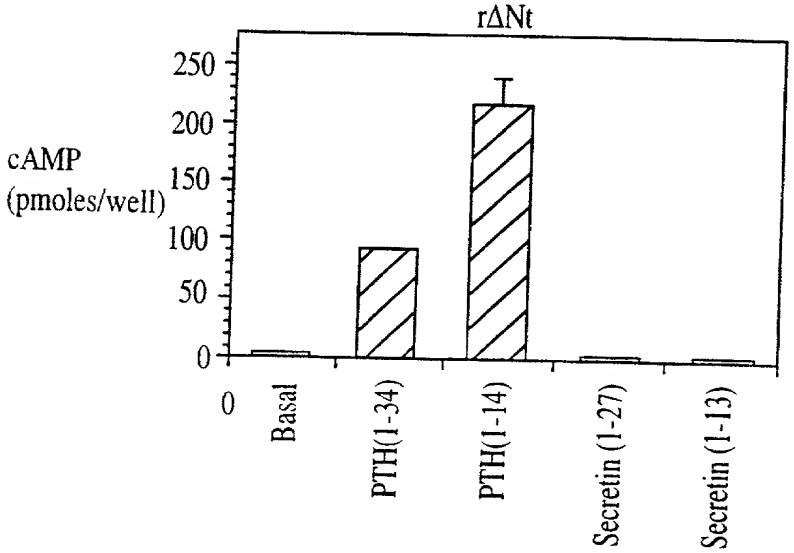

FIG. 6B

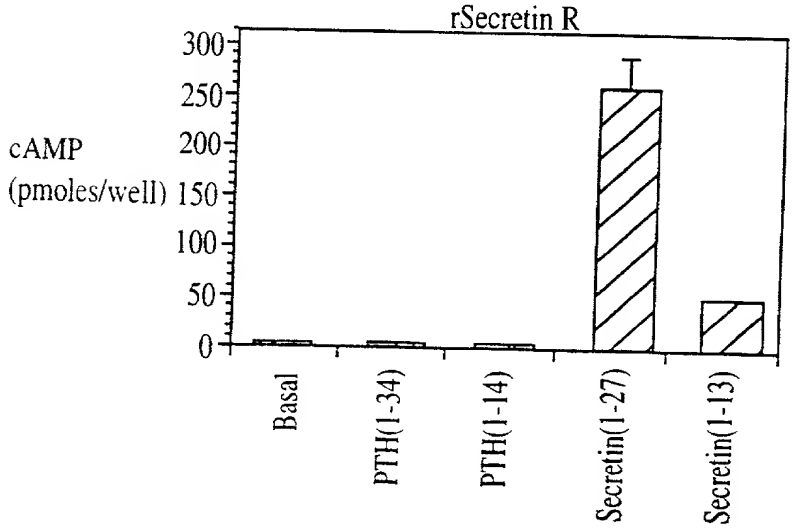

FIG. 6C

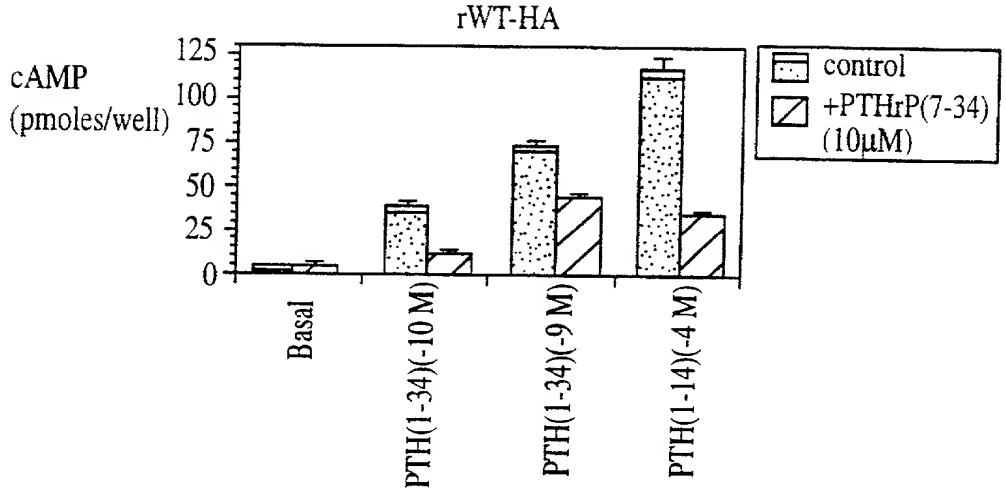
rWT-HA
cAMP
(pmoles/well)
125
100
75
50
25
0
control
+PTHrP(7-34)
(10μM)
Basal
PTH(1-34)(-10 M)
PTH(1-34)(-9 M)
PTH(1-14)(-4 M)

FIG. 7A

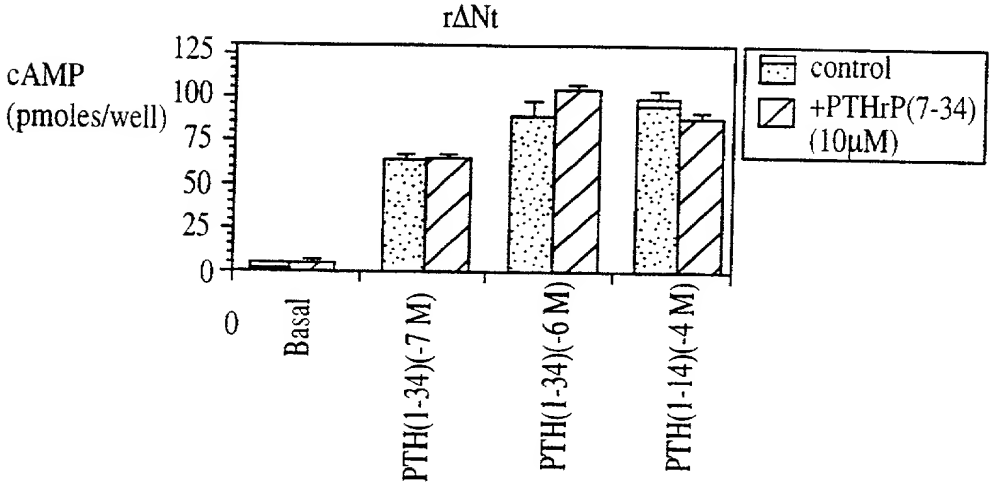
rΔNt
cAMP
(pmoles/well)
125
100
75
50
25
0
0
control
+PTHrP(7-34)
(10μM)
Basal
PTH(1-34)(-7 M)
PTH(1-34)(-6 M)
PTH(1-14)(-4 M)

FIG. 7B

Declaration for Patent Application

MGH Ref. 1454.0
SKGF Ref. 0609.4730000

As a below named inventor, I hereby declare that:

My residence, mailing address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter that is claimed and for which a patent is sought on the invention entitled: **PTH Receptor and Screening Assay Utilizing the Same**,

the specification of which is attached hereto unless the following box is checked:

☒ was filed on June 29, 2001;
as United States Application Number or PCT International Application Number 09/869,565; and
was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56, including for continuation-in-part applications, material information that became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or (f), or § 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or § 365(a) of any PCT international application, which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed
PCT/US98/27862	_____	31 December 1998	☐☒ Yes ☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)	
_____	_____	_____	☐ Yes ☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)	

Send Correspondence to:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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(Supply similar information and signature for subsequent joint inventors, if any)	SKGF Rev 5/16/01 mac